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APPLE LEAF SPOT

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(WITH PLATE XVII)

The following paper is a report upon the cause and nature of apple leaf spot and includes data and discussion in regard to inoculation experiments with various fungi, studies on different forms of *Sphaeropsis malorum*, and experimental work on time of infection, influence of cultivation and methods of control.

Apple leaf spot appears upon the leaves soon after they have unfolded from the bud. At first the spots are quite small and have a purplish tint. They enlarge rapidly, soon attaining a diameter of 2 to 8 millimeters, and the color soon changes to a dull brown with a darker marginal zone. The spots are circular in outline. As they become older a secondary growth may develop at the margin finally producing a somewhat irregular dead brown area in which the outline of the original spot can be readily recognized. The appearance of the disease at this stage has given rise to the name "Frog Eye" spot. Spotted leaves fall from the trees early in the season and in extreme cases almost entire defoliation may occur six to eight weeks before the time of the normal leaf fall.

Various agencies have been credited with causing the disease. Among these are spray injury [1], intense sunlight following showers, [1] frost, [2] and a number of different fungi.

Lamson [3] and several other early investigators held that *Coniothyrium pirina* (Sacc.) Sheldon, formerly known as *Phyllosticta pirina*, was the cause of the trouble. Stewart [4] found *Phyllosticta limitata* Pk. responsible for an outbreak of leaf spot on Long Island. In 1902

[1] Stewart, F. C. and Eustace, H. F. New York Agr. Exp. Sta. Bul. 220 : 225–233. 1902.

[2] Stone, George E. and Smith, Ralph E. Mass. Agr. Exp. Sta. Rep. 15 : 32–34. 1902.

[3] Lamson, H. H. N. H. Agr. Exp. Sta. Bul. 65 : 106–107. 1899.

[4] Stewart, F. C. N. Y. Agr. Exp. Sta. Rep. 14 (1895) : 545–546. 1896.

Clinton [1] reported *Sphaeropsis malorum* Pk. as the common cause of leaf spot in Illinois and in 1903 made a similar report from Connecticut [2].

Scott and Rorer [3] seem to have been the first to make an extended study of apple leaf spot. They made cultural studies of diseased leaves from Arkansas and other states and found that *Coniothyrium pirina* (Sacc.) Sheldon, *Sphaeropsis malorum* Pk. one species each of Coryneum, Hendersonia, and Alternaria, and two species of Pestalozzia could be isolated from the spots. Inoculation experiments were made with the first five of these but positive results were obtained only with *Sphaeropsis malorum*. They concluded that the leaf spot of apple found in the middle West was due to this fungus. They also believed the leaf spot of the Eastern States to be due to the same cause.

CAUSE OF LEAF SPOT

The conclusions of Scott and Rorer were in direct disagreement with those of Lamson who had reported on the disease from New Hampshire. This difference in results, together with the seriousness of the disease in that state led to a study of possible causes. The work was begun in the summer of 1908 by I. M. Lewis.[4] *Sphaeropsis malorum*, *Coniothyrium pirina*, *Coryneum foliicolum* and a species each of Alternaria and Fusarium were isolated from the affected areas. Spots that were still young and purple quite generally gave pure cultures of *Sphaeropsis malorum*, indicating that this fungus was probably the primary cause of the trouble, but pycnidia of *Coniothyrium pirina* were quite often obtained. Inoculation experiments were made with spores from pure cultures of the several fungi mentioned above. The inoculations in these and in the other experiments reported in this paper were made in the afternoon between four and six o'clock and the spores were placed in sterile water and applied by means of an atomizer. Check branches were sprayed with water only. Four applications were made in 1908, the first on August 1. None of the inoculated leaves showed any decided increase in spots over the checks. Although the leaves used were those that had developed latest in the season, it was thought at the time that the maturity of the leaves might be responsible for the uniformly negative results. The data given later (See page 187) on time of infection would indicate, however,

[1] Clinton, G. P. Ill. Agr. Exp. Sta. Bul. 69 : 192–193. 1902.

[2] Clinton, G. P. Conn. Agr. Exp. Sta. Rep. **1903** : 298. 1903.

[3] Scott, W. M. and Rorer, J. B. U. S. Dept. Agr., Bureau of Plant Industry, Bul. 121 : 47–54. 1908.

[4] Lewis, I. M. N. H. Exp. Sta. Rep. **20** : 365–369. 1908.

that this was not the case. It is possible that the weather conditions were responsible for the results obtained.

The work begun by Lewis in 1908 was continued the following spring. Inoculations were made with *Coniothyrium pirina* and *Sphaeropsis malorum* on apple seedlings in the greenhouse. Other seedlings were sprayed with sterile water as checks. No spots developed on the checks nor on the seedlings inoculated with Coniothyrium. Those inoculated with Sphaeropsis showed a great variation. Some were entirely free from spots, others had a few spots, while one had its leaves thickly spotted. This variation in results with Sphaeropsis may have been due to a difference in natural susceptibility, as the origin of the seedlings was unknown and different varieties show a great variation in resistance to the disease. It is interesting to note in this connection that Lewis[1] of the Maine Experiment Station obtained only negative results in inoculation experiments with *Sphaeropsis malorum* and other leaf spot fungi on apple seedlings.

Similar inoculation experiments were made on Baldwin trees in the orchard. Beginning May 11, and extending to the last of July inoculations were made twice a week with Coniothyrium and Fusarium. Only negative results were obtained. Considerable difficulty was found in getting the Sphaeropsis cultures to produce spores at the time they were wanted and early inoculations mere not made with this fungus. Several sprayings were made in June with Sphaeropsis. Notes taken the latter part of July showed that, although some of the sprayed leaves were thickly spotted, the average number of spots on the inoculated leaves was not greater than that of the checks. Negative results were also obtained from inoculations with Alternaria.

The summer's work was not conclusive, but the negative results obtained from the large number of inoculations made with Conithyrium and Fusarium indicated that these could hardly be of importance in the production of the disease.

During the winters of 1908–09 and 1909–10 a large number of cultures of *Sphaeropsis malorum* were obtained from spots on apples. These showed a great variation in the nature of their growth and in the time required for spore production. This fact and the report of Walker[2] of two forms of *Sphaeropsis malorum* in Nebraska suggested the possibility that the results obtained in the inoculation work with

[1] Lewis, C. E. Inoculation experiments with fungi associated with apple leaf spot and canker. Phytopathology **2**: 49–62. 1912.

[2] Walker, Leva Belle. A new form of Sphaeropsis on apples. Neb. Exp. Sta. Rep. **21**: 35–44. 1908.

Sphaeropsis might have been modified by the nature of the fungus in culture.

Accordingly a study was made of *Sphaeropsis malorum* from different sources. Three strains or types were found that were particularly distinct. The first had broadly conical pycnidia that were definitely papillate and produced oblong spores measuring 12 x 26–38 microns (See Figs. 1 and 2). The second strain produced egg-shaped spores measuring 14 x 23 microns. Its pycnidia were nearly spherical, very slightly papillate and sometimes showed a multilocular tendency, the inner walls extending in at various places, giving the cavity a somewhat corrugated appearance. The third was characterized by a distinctly multilocular pycnidium, the wall extending in at various places to form a number of pockets, all of which opened into a common cavity (See Figs. 3 and 4). The spores of the third were like those of the first and the external outline of the pycnidium like that of the second. All three forms were ostiolate.

The above descriptions apply particularly to the conditions as found on bean cultures and the characteristics given have been maintained throughout a great many generations of cultures on that medium. When transferred from cankers to beans the second strain sometimes produced both large and small spores. When later transferred to apple twig cultures it often became definitely multilocular and again produced both large and small spores but a smaller number of the former than in the bean cultures. The per cent of large spores was not great in either case. The strain kept its small-spored condition when grown on apples. The first strain retained its characteristics when grown under these various conditions. The third strain sometimes lost its multilocular condition when transferred to apples. This was also true when grown in apple twig cultures. When transferred from apple twig cultures back to bean cultures the multilocular condition was partially or entirely regained.

Cankered limbs were examined from various sections of the state for different forms of Sphaeropsis. The first strain was the most common on these, but several had pycnidia of the second form. No extended study was made of pycnidia as they occurred on the foliage but the cultures obtained from leaf spots always produced pycnidia of the first form. Only the first form of pycnidium was found on the fruit of apples in the field and the same was true of quinces. All three forms were obtained in cultures made from spots on apples, but the first was much more common than either of the others. The three strains were kept growing in culture on bean pods and inoculation experiments made on the foliage and fruit.

Each of three young Baldwin trees was covered by a closed glass frame. The frames were 20 by 20 inches and 3½ feet high. The trees were approximately alike. One of these trees was sprayed with spores from the first strain of Sphaeropsis, another with spores from the second and a third with sterile water. The cultures of the second strain came from a canker, those of the first from an apple. The water in which the fungous growth had been placed was examined before being used to see that it contained an abundance of mature spores. Beginning with May 16 five sprayings were made at intervals of four to seven days. Notes taken June 13 showed the following results:

Total Number of Leaf Spots

First strain, 105 Second strain, 7 Checks, 3

Similar inoculations were made in the open on large Baldwin trees in the same orchard. Beginning on May 9 and extending to June 20, thirteen different inoculations were made at fairly regular intervals. A different set of twigs was sprayed each time, none of the leaves having more than one application. A comparison was made of the results obtained from early and late inoculations. The leaves were apparently as susceptible at the middle of June as early in May. The following table is a summary of the results obtained with the different strains of Sphaeropsis as shown by notes taken July 27:

Percent of Leaves Spotted

First strain, 33.6 Second strain, 26.8 Checks, 22

The two sets of data seem to show conclusively that the spores from the first form of Sphaeropsis are more capable of producing leaf spot than those of the second. This data agrees with the previously mentioned fact that only the first form was obtained in cultures from leaf spots.

Tests were made with the two strains to determine their power to produce rot on the fruit. Baldwin apples were inoculated while still on the tree. The inoculations were made by forcing a spore bearing needle into the apple. Other punctures were made with a sterile needle. Forty inoculations with each fungus and forty check punctures were made at each application. Inoculations were made on September 5, 16, and 28. At the time of the first treatment the apples were quite green, when the last one was made they were nearly mature. Notes taken on the stored apples on November 24 showed the following results:

Results from Inoculations on Fruit

Date of inoculation	Number of cases of black rot developed		
	First strain	Second strain	Check
Sept 5	19	4	4
" 16	22	18	3
" 28	16	21	2

The second form was apparently able to produce rot on nearly mature apples as well as the first, but was without effect upon the green fruit.

The apples were kept in cellar storage and their condition noted at various times. The rot developed more rapidly on the apples inoculated with the first strain. On January 17 several of these had produced pycnidia with mature spores while no spore-bearing pycnidia could be found on the apples inoculated with the second strain. On March 7th there were five times as many apples of the former lot that had spore-bearing pycnidia as there were of the latter. The apples inoculated with the large-spored strain always developed pycnidia producing large spores. Those inoculated with the small-spored strain on September 5 produced large-spored pycnidia; those inoculated on the later dates in most cases produced small-spored pycnidia but pycnidia bearing both large and small spores were not uncommon. That the apples receiving inoculations with the second type of fungus on September 5 should have produced the first type of pycnidium is not surprising since the rot on these did not exceed that on the checks, and may have been due to chance inoculation.

The fact that the second strain was not vigorous on the foliage and was unable to infect green apples, that it produced rot more slowly on mature fruit and developed its pycnidia later and in smaller numbers than the first strain, would indicate a physiological difference in the two strains. The fact that the first strain always, and the second generally, retained its peculiar spore form when grown on unripe apples furnishes further evidence of the permanance of the structural differences in the two strains.

The third strain was also tested under various conditions in comparison with the first strain. Spraying inoculations were made on the foliage of young Baldwin trees in the greenhouse. Only one strain was applied on a particular tree. The number of leaves sprayed with each fungus was approximately the same. The spores were taken from cultures on beans. The solutions were examined before using, as previously described. Check trees were sprayed with sterile water. Sprayings were made March 26, 27, 28, 29 and April 1 and 2. Notes taken April 27 showed the following results:

Number of Spotted Leaves

First strain		Third strain		Check
From leaves	From apples	From apples	From quinces	
23	20	0	1	0

Inoculations were also made in the open on Baldwin trees in the orchard. The work was begun as soon as the buds opened. Sprayings were made May 3, 4, 7, 10 and 14. Notes taken on May 27 showed that the leaves inoculated with the third strain were free from spots, while about 10% of those inoculated with the first strain were spotted. The number of spots produced by the first strain was again greater where cultures from leaves were used than where those originally taken from the fruit were applied. Pure cultures of the first strain were obtained from the leaves inoculated with that fungus.

Inoculations were also made on apples with the three cultures mentioned above. The third strain produced rot more slowly than the first and the cultures of the first strain from leaves showed greater vigor on the fruit than those originally obtained from the fruit.

The results obtained would indicate that the third strain has but little power to produce leaf spot and is lacking in general vigor.

The inoculation experiments and cultural studies with *Sphaeropsis malorum* from various sources indicate that several strains of the fungus exist, having different degrees of vigor in the production of disease and more or less distinctive structural characteristics. The peculiarities in the pycnidia of some of these strains have been modified by cultural conditions but the original tendencies have been retained on various media and during several months of laboratory study.

The parasitism of *Sphaeropsis malorum* seems especially interesting from the physiological standpoint, since the fungus attacks such widely different host materials as the fruit, stem and foliage. Of these three the leaves are apparently least susceptible, the fungus making but a scant growth on them, and fruiting only after a long period of development. It would be entirely in keeping with the responsiveness of the fungus for several generations of growth on one host material to produce more or less permanent physiological and even structural modifications in the parasite.

TIME AND MANNER OF INFECTION

The time of infection is a matter of importance in the control of leaf spot and a factor to be considered in inoculation experiments. Failure to secure results with inoculations made in August as previous-

ly mentioned and similar failures in July by Lewis[1] in Maine seemed to indicate that the foliage is not susceptible to the disease late in the season. The following records show that if this lack of susceptibility exists it is at most only partial.

The spring and early summer of 1910 was characterized by a large number of gentle showers. As a result of this condition the relation of cankers to leaf spot was made particularly evident. The foliage in a cone-shaped area beneath affected limbs became thickly spotted while the leaves in other parts of the tree were almost free from spots. One tree was found in which this condition was particularly striking. Two large limbs in the top were dead and the main branches as well as the twigs were practically covered with fruiting Sphaeropsis. The pycnidia were of the first type. The leaves beneath these spore-bearing limbs became thickly spotted, while those at a distance were practically uninfected. The striking condition was noted early in the season and it was decided to follow the development of the spots throughout the summer. On July 1 twenty twigs that were at various points beneath the cankered limbs were marked and a count made of the number of leaves and the number of spots. Similar counts were made of the same twigs on August 10 and August 25. The number of leaves on the twigs more than doubled between the first two dates. Between August 10 and August 25 there was but very slight change in the number of leaves; some of the twigs produced a few new ones, but others lost a few. It is probable that the leaves that dropped were the worst spotted ones. The following table gives a summary of the results:

Average Number of Spots per Leaf

July 1	August 10	August 25
.66	4.2	5.2

It is apparent that the spots must have have developed more rapidly in July than earlier in the year and when we note that the period between the last two dates is only about one-third as great as that between the first two it is evident that the increase per day was nearly as great after August 10 as before. The leaves on the more distant parts of the tree remained practically free from spots throughout the season. The results make it quite conclusive that vigorous spores of Sphaeropsis may produce leaf spot throughout the middle of the

[1] Lewis, C. E. Phytopathology **2**: 49–62. 1912.

summer and would indicate that negative results obtained from inoculations made late in the season must be due to unfavorable weather or to the character of the cultures used.

The above data on leaf spot emphasizes the importance of cankers in the production of the disease. It seems probable that a large part of the late infection in orchards is produced by spores from cankers.

EFFECT OF CULTIVATION

An opportunity to secure data upon the effect of cultivation was found in an experimental Baldwin orchard. The orchard consisted of about 300 trees and was divided into ten plots, each plot receiving a different treatment. Notes were taken in the fall of 1910 when the experimental work had been in progress three years. Data were secured on ten twigs near the ground and ten in the top of each of four trees in each plot. The number of spots was thus counted on about one thousand leaves from each plot. There was apparently no difference in leaf spot between the upper and lower branches. The plots receiving various fertilizer treatments showed little variation in leaf spot. The manner of cultivation likewise seemed unimportant. The only marked contrast in leaf spot was between the sod and the cultivated plots. The average number of spots per leaf on the former was .79 on the latter .47. The trees had been treated alike in every other respect and were of equal vigor when the experimental work was begun ; it was, therefore, evident that cultivation had reduced the disease almost one-half. This reduction was probably partly due to the fact that the leaves were plowed under on the cultivated plots, but the lack of general vigor in the trees on the sod plots was apparently partly responsible for the difference.

METHODS OF CONTROL

What has already been said in regard to cankers and cultivation suggests two things of importance in control ; cutting out cankers and plowing under the leaves. A partial remedy may also be found in spraying. In the summer of 1908 spraying experiments were made with various fungicides in a McIntosh orchard in Deerfield, N. H. Five sprayings were made, the first May 15, just before the flower buds opened, the second May 27, after the blossoms had fallen, and the others June 10, June 18 and July 30. The following results were obtained :

The Control of Leaf Spot by Spraying

Fungicide	Leaves spotted
None	97 per cent
Lime-sulphur, self-boiled (9 lbs. lime : 6 lbs. S.: 50 gals. water)	21 " "
Lime-sulphur, self-boiled (15 lbs. lime : 10 lbs. S.: 50 gals. water)	21 " "
Lime-sulphur, commercial (1 gallon to 25 gals. water)	26 " "
Lime-sulphur, commercial (1 gallon to 50 gals. water)	33 " "
None	95 " "
Bordeaux mixture, Eagle (1 gallon to 24 gals. water)	26 " "
Bordeaux mixture, (3-3-50)	26 " "
Bordeaux mixture, (4-4-50)	30 " "

It is apparent that either Bordeaux mixture or lime-sulphur is an efficient remedy for the disease. Under New Hampshire conditions Bordeaux often injures the leaves of Baldwins and other varieties, producing spots very similar to the fungous leaf spots. This is particularly true when showers follow soon after the application of the fungicide. Lime-sulphur seems on the whole, to be the most satisfactory fungicide for the control of the disease.

CONCLUSIONS

The following conclusions seem to be justified by the data reported above.

1. The leaf spot of apples as it occurs in New Hampshire orchards may be produced by *Sphaeropsis malorum* Pk. and is probably largely due to that fungus.

2. Several strains of *Sphaeropsis malorum* may be obtained, varying in general vigor and in power to produce diseased conditions. The large-spored form, with single-loculed, ostiolate pycnidia is largely responsible for the production of leaf spot.

3. Infection may occur from the time the leaves unfold till the last of August.

4. Cultivation, spraying and the removal of cankers are important in the control of the disease.

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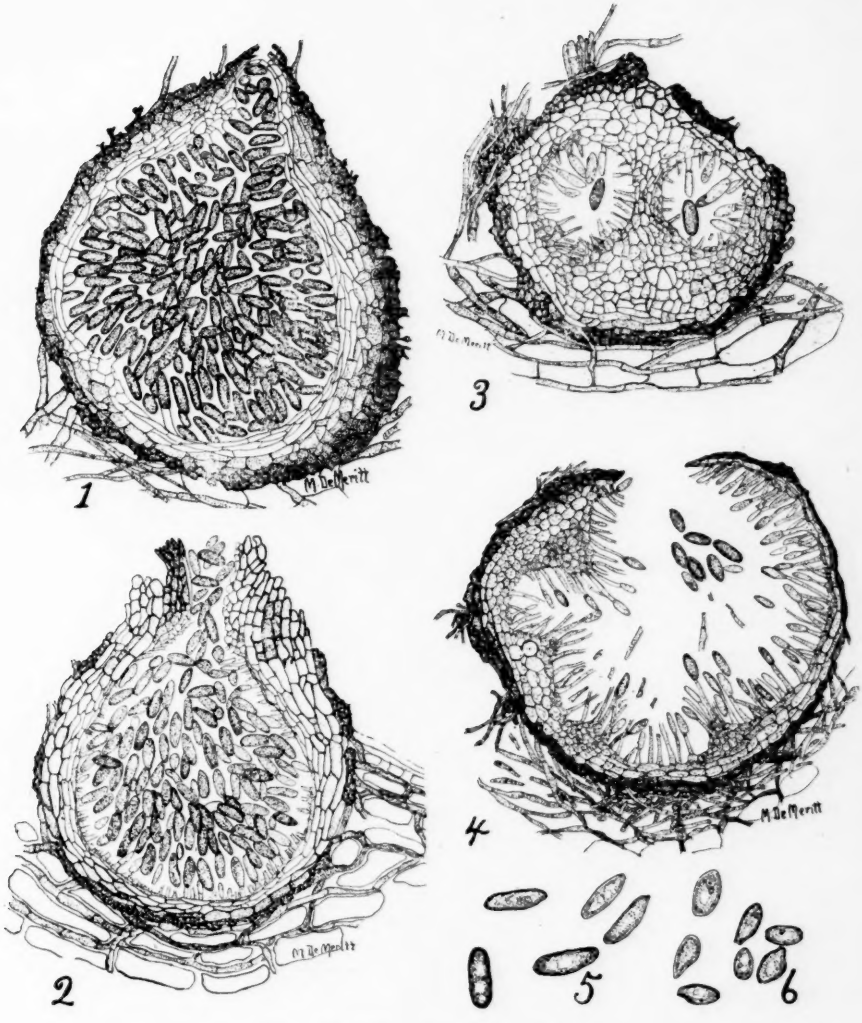

Plate XVII.—Apple Leaf Spot

FIG. 1. Large-spored, single-loculed pycnidium of *Sphaeropsis malorum* from bean culture.

FIG. 2. Large-spored, single-loculed pycnidium from an apple.

FIGS. 3 and 4. Large-spored multilocular pycnidia from bean culture.

FIG. 5. Oblong spores of strain 1 of *Sphaeropsis malorum*.

FIG. 6. Egg-shaped spores of strain 2 of *Sphaeropsis malorum*.

INFECTION EXPERIMENTS WITH THIELAVIA BASICOLA ON GINSENG

J. ROSENBAUM*

(WITH PLATES XVIII AND XIX)

One of the most common and serious diseases affecting ginseng has been attributed to *Thielavia basicola* Zopf. While several workers in different parts of the United States have observed the fungus associated with the disease, no definite inoculation experiments on this host have ever been reported. In fact, in reviewing the literature dealing with this fungus, it is surprising how little has actually been accomplished toward establishing its pathogenicity for the plants on which it has been observed to occur.

The purpose of this paper is to report inoculation experiments with this fungus obtained from different hosts. As will be seen most of the work was necessarily done with ginseng seedlings, especially as regards the carrying out of Koch's "Rules of Proof."

Mr. W. W. Gilbert of the United States Department of Agriculture kindly furnished cultures of this fungus isolated from cotton as well as from tobacco. Another culture was isolated by the writer from ginseng seedlings.

HISTORICAL

Berkeley and Broome[1] in 1850 first described the fungus, reporting it as a probable parasite at the base of the stems of the pea and other plants. They wrote "it is either destructive on the plant on which it grows or is developed in consequence of previous disease."

Twenty-five years later Zopf[2] gave the first complete description of the different stages of the fungus. It is reported by him as injuring species of Senecio as well as a number of leguminous plants. He concludes that it is a true parasite and calls the injuries of the root which it produces "Wurzelbräune."

Sorauer[3] found the fungus on Cyclamen and comes to the conclusion that it leads a saprophytic, as well as a parasitic existence, since he

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[1] Berkeley, M. J. and Broome, C. E. Notices of British fungi. Ann. Mag. Nat. Hist., s. II. 5 : 461. 1850.

[2] Zopf, W. Über die Wurzelbräune der Lupinen, eine neue Pilzkrankheit. Zeitschr. Pflanzenkr. 1 : 72–76. 1891.

[3] Sorauer, P. Über die Wurzelbräune der Cyclamen. Zeitschr. Pflanzenkr. 5 : 18–20. 1893.

found it also on leaf molds. He states that very often it occurs in the soil without doing any injury.

Peglion[1], who first found the fungus on tobacco, reaches the conclusion that an excess of water determines whether the fungus becomes very destructive.

Galloway[2] in studying the diseases on violets also concludes that a water logged condition of the soil is intimately related to the severity of the disease.

None of these investigators appear to have attempted infection experiments. Aderhold[3], one of the few men making infection experiments on a number of hosts, was able in some cases to produce the disease but only at the crown of the plants. In any case, he seems to agree with the majority of workers that certain unfavorable conditions are factors in determining its parasitic nature. Clinton[4] has no doubt of the parasitic nature of the fungus "under certain conditions," but reports no definite infection experiments.

Gilbert[5], whose observations extended over a period of four years, performed a number of inoculation experiments. Infection was obtained in every instance, while the checks remained healthy. From these experiments the author has no doubt of the parasitic nature of the fungus.

Massee[6] in a recent paper describes a disease of sweet peas, asters and other plants caused by this fungus. He carried out some infection experiments and comes to the conclusion that the fungus must exist for some time as a saprophyte on humus or decaying vegetables in order to be able to infect the host plant directly.

In summing up, we may say that most workers agree that the fungus is parasitic. Some claim, however, that external conditions determine whether it becomes very destructive in any given locality.

In the opinion of the writer, who has been working with the fungus for the past two summers, while external conditions, such as excessive

[1] Peglion, V. Marciume radicale delle piantine di tabacco, causato dalla Thielavia basicola Zopf. Atti Reale Accad. Lincei **5**: 52–56. 1897. Reprint Cent. Bakt., Abt. 2, **3**: 580–583. 1891.

[2] Galloway, B. T. Commercial Violet Culture. pp. 170–174. New York. 1899.

[3] Aderhold, R. Impfrersuche mit Thielavia basicola Zopf. Ark. k. Gsndhtsamt., biol. Abt. **4**: 463–465. 1905.

[4] Clinton, G. P. Root-rot of tobacco. Conn. Agr. Exp. Sta. Ann. Report **30**: 342–368. 1907.

[5] Gilbert, W. W. The root-rot of tobacco caused by Thielavia basicola. U. S. Dept. Agr., Bur. Pl. Ind. Bul. 158. 1909.

[6] Massee, G. A disease of sweet peas, asters and other plants. Bul. Miscell. Inform. Kew. **1912**: 44–52. *pl. 1.*

water, lack of aeration, heavy manuring, susceptibility of the plants, etc., no doubt influence the severity of the infection, as they do in the case of most soil organisms, repeated inoculation experiments, especially in the case of ginseng plants, prove that it is a true parasite capable of producing disease on direct inoculation, having a definite period of incubation and forming a typical lesion.

OCCURRENCE

The fungus is very widely distributed and has been associated with a wide range of hosts belonging to entirely different families. Families containing species known to be attacked by this fungus are: *Cruciferae*, *Leguminosae*, *Violaceae*, *Oxalidaceae Malvaceae*, *Araliaceae*, *Primulaceae*, *Hydrophyllaceae*, *Scrophulariaceae*, *Bignoniaceae and Compositae*.

It has been reported from Germany, Russia, Belgium, England, Italy and Korea. In the United States it has been reported from a dozen or more states. In the herbarium of the Department of Plant Pathology, Cornell University, there are specimens of diseased ginseng from the following states: New York, Pennsylvania, Maryland, Ohio, Vermont, Wisconsin, Michigan, Minnesota, Indiana, Illinois, and Nebraska. It has been observed to attack the ginseng plant in soils of such widely different physical characters as lake shore sand and heavy clay soil. In many cases the disease seems to be just as severe on new land as on soil which has previously grown several crops. In the case of ginseng, the fungus is able to attack plants of all ages, being most severe on seedlings, where it is known by the growers as "fiber-rot" or "end-rot." It not only attacks the roots of seedlings and older plants, but has been found causing cankers an inch or more in length on the stems. Most commonly these occur at the point where the stem emerges from the ground. These cankers at first cause a discoloration, followed by a drying and splitting of the stem in a longitudinal direction, allowing it to break over.

ISOLATION AND CULTURAL CHARACTERS

For a long time there was difficulty in isolating the fungus and getting it to grow in pure culture, though numerous chlamydospores are always present on the roots of the seedlings or on the stem cankers. Bacteria in all cases seemed to overrun the poured plates before the spores germinated. Other workers have had similar cultural difficulties. Gilbert states that finally he was able to obtain the fungus in pure culture by growing it on a beef agar medium to which a 2% solution of sodium nitrate was added. This medium was tried, but for some reason no greater success was obtained. The following

method was finally used and the fungus repeatedly isolated from diseased seedlings or cankers on the stem. The tissue affected is washed for ten minutes in a solution of 1-1000 mercuric chloride. It is then thoroughly rinsed with distilled water and a small piece containing the chlamydospores is crushed in a drop of sterile water in a petri dish. Dilution plates are then made from this, using ordinary potato agar to which is added one drop of a 50% solution of lactic acid. While the acidity of the medium is not sufficient to entirely prevent the growth of bacteria, they will grow but slowly. At the same time such acidity will not prevent the growth of the fungus. As soon as the medium is hardened, the poured plates are examined under the microscope and single chlamydospores marked. At the end of five days, the chlamydospores will have grown far enough to make transfers possible, but the surface is apt to be more or less covered with bacterial colonies. In making the dilution plates, the chlamydospores quickly sink to the bottom while the bacterial colonies are more evenly distributed, being caught on the surface by the hardening agar. One of the germinated chlamydospores which had been previously marked, is selected and the whole block of agar, about 1 cm. square, carefully cut out with a sterile scalpel and inverted on a flamed slide. What originally corresponded to the lower surface of the agar in the plate is now the upper surface of the block. This block has the germinated chlamydospore on the upper surface and bacterial colonies mostly imbedded on the lower surface. By using a sterile platinum needle, plantings are made by gently scraping the top of the block and transferring fragments of mycelium and spores to tubes of potato agar or other sterile medium. In making these transfers care must be taken not to push the needle down to the lower surface of the block. The same method of procedure has proven successful in obtaining pure cultures of other fungi where the surface of the colony was run over by bacteria.

The fungus from tobacco, cotton and ginseng was grown on several different media. At the end of three weeks the growth of the fungus from the different sources proved in all cases to be the same (Plate XIX). The best growth was obtained on hard potato agar containing 2.5% agar and 2% glucose. An abundance of both endoconidia and chlamydospores were produced on this medium.

INOCULATIONS

The cultures used for inoculation were isolated from three different hosts, namely, cotton, tobacco and ginseng, designated in the order named as *c.* *t*, and *g*.

Inoculations of seedlings. Ginseng seeds were treated with a 10% solution of formalin for five minutes and washed thoroughly in distilled water. Part of them were placed on a moist filter paper in a preparation dish and part on sterile quartz in a similar dish. In two weeks the seed had germinated and had grown to considerable size. At this time they were sprayed with water containing the chlamydospores of culture *t*. Numerous checks of seed grown both on filter paper and quartz were given the same treatment with the exception that no spores were added to the water used for spraying. At the end of ten days all seedlings sprayed with the chlamydospores showed the characteristic rusty color at the tip of the root and were entirely devoid of root hairs. The check cultures remained healthy, showing an abundance of root hairs. (Plate XVIII, Fig. 4).

When the tips of these seedlings were crushed in a drop of water they were found to be covered with endoconidia and chlamydospores. Re-isolations were made from these inoculated seedlings and the fungus was again obtained in pure culture. Similar results were obtained when inoculations were made on ginseng seedlings with cultures *g* and *c*.

A similar set of inoculations was carried out with cultures *t*, *g* and *c* on tobacco seedlings grown on moist filter paper and sterilized quartz in preparation dishes. In all cases, ten or fifteen days after inoculation the seedlings bent over and wilted while the checks remained healthy.

Inoculation of ginseng in the soil. A row of twenty-four seedlings growing in the garden were inoculated with culture *g*. In making this inoculation, the soil was removed from the plants so as to expose part of the root. Pieces of the agar containing spores of the fungus were placed on the exposed surface of the roots which were immediately covered with soil. The rows on each side were left as checks. At the end of ten days some of the seedlings began to fall over. They were taken up carefully and minute black spots were found at the place of inoculation, which when examined proved to be made up of numererous chlamydospores of *Thielavia basicola*. At the end of three weeks only one seedling remained standing in the inoculated row, while in the check rows no such wilting had taken place (Plate XVIII, Fig. 2). Re-isolations were made from these infections and the fungus was again obtained in pure culture.

Attempts to inoculate the roots of older ginseng plants in the soil, without first pricking the root, were unsuccessful. When the roots were injured, however, there was no difficulty in getting infection with cultures *t*, *g* or *c*. The spots on the larger roots have a more or

less jet black center, due to the chlamydospores, surrounded by a discolored rusty area. Two such roots, inoculated with culture *g*, may be seen in Fig. 1, of Plate XVIII.

Inoculations on stems. While the fungus is most commonly found attacking the roots of plants, specimens of ginseng stems attacked by this pathogen were collected in New York and Michigan. Culture *g*, when inoculated on a two year old ginseng stem by pricking the stem with a sterile scalpel, produced black cankers at the end of fifteen days. (Plate XVIII, Fig. 3).

MORPHOLOGY OF THE FUNGUS

The illustrations in Plate XIX are camera lucida drawings of the fungus from the different hosts. From these as well as from careful measurements of chlamydospores, mycelium and endoconidia, no morphological difference of specific or even varietal value could be detected.

CONCLUSIONS

1. From comparison of cultures from different hosts on various media, from infection experiments and from measurements of spores and mycelium it appears that the forms of *Thielavia basicola* Zopf found on the three different hosts, cotton, tobacco and ginseng, are identical.

2. While infection without previous injury to the ginseng or tobacco is always obtained in the case of young plants, this is apparently not the case where older plants are used.

3. The fungus is able to attack the aerial as well as underground parts of the plant; at least this proved to be the case where ginseng is concerned.

The writer is indebted for many suggestions to Professor H. H. Whetzel under whose directions this work was done.

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EXPLANATION OF PLATE XVIII

FIG. 1. Two-year old roots inoculated with a culture of Thielavia isolated from ginseng. The jet black spots are made up of numerous chlamydospores.

FIG. 2. A single ginseng seedling standing in an inoculated row as compared with the rows on each side not inoculated. Photograph three weeks after inoculation.

FIG. 3. Two stems from two year old roots inoculated with a culture from ginseng. Black cankers are produced by such inoculations.

FIG. 4. Ginseng seedlings inoculated with Thielavia culture from tobacco. The five seedlings to the left inoculated—others healthy. Note the black tip and lack of root hairs in the case of those inoculated.

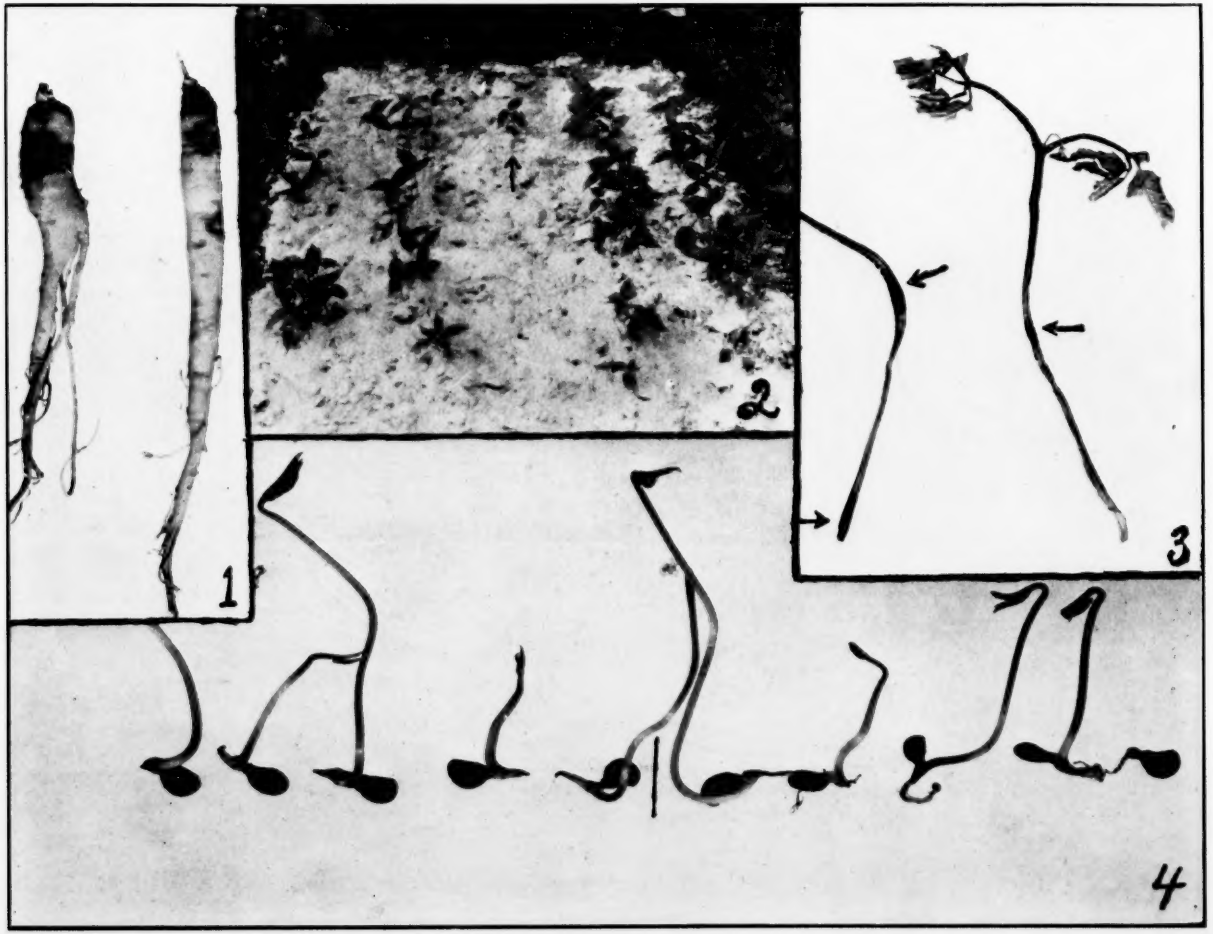

Plate XVIII.—Infections with Thielavia on Ginseng

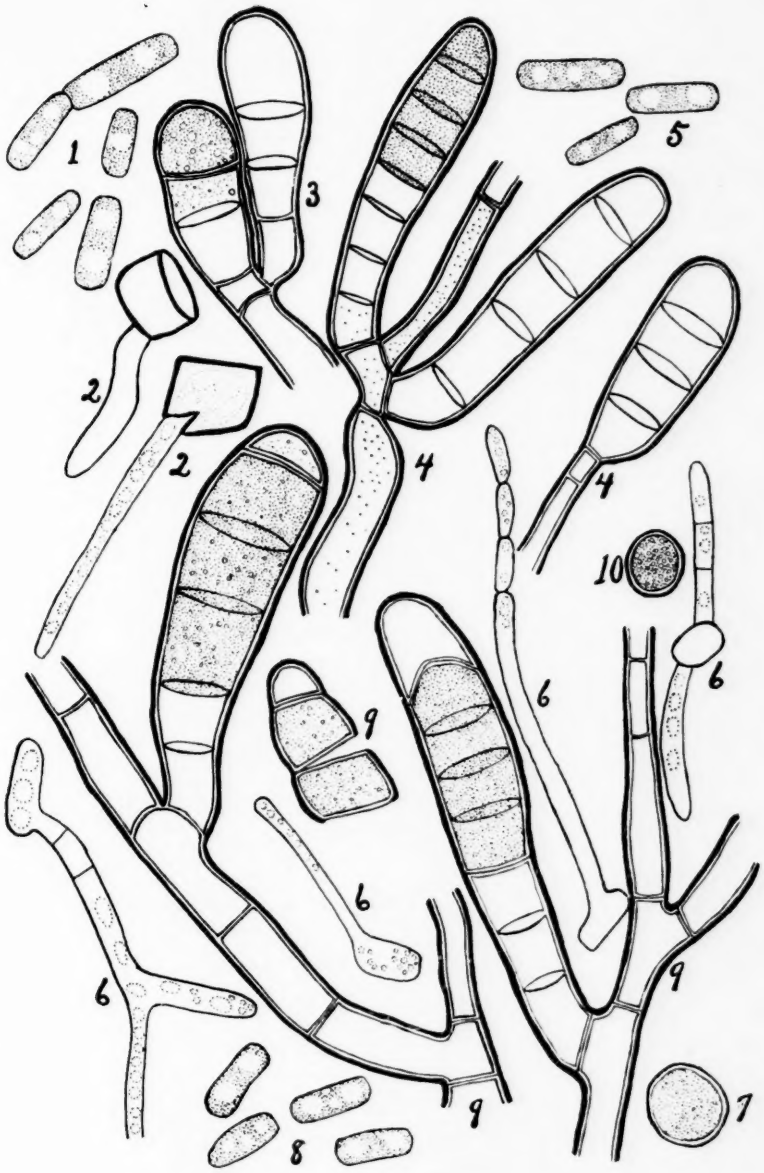

Plate XIX.—Thielavia from Different Hosts

FIGS. 1, 5 and 8. Endoconidia from cotton, ginseng and tobacco, respectively.

FIG. 2. Chlamydospores. 36 hours in distilled water.

FIGS. 3, 4 and 9. Chlamydospores from cotton, ginseng and tobacco, respectively.

FIG. 6. Endoconidia. 24 hours in distilled water.

FIGS. 7 and 10. Single chlamydospores from tobacco and ginseng.

CULTURING OF PARASITIC FUNGI ON THE LIVING HOST

I. E. MELHUS

(WITH PLATE XX AND TWO TEXT FIGURES)

It has become a comparatively easy matter to grow a large number of parasitic fungi on artificial media. This achievement has had a marked influence not only on the methods of investigation but also on the teaching of a variety of subjects where plant parasites are concerned. It is no longer necessary to imagine a living host infected with a given fungus from dried specimens that may have been collected several years previously; instead, cultures of living fungi growing either on artificial media or on the living host should be at our disposal.

Sufficient work has been done with pure culture methods on artificial media to show quite clearly their limitations as well as the desirability of culturing a parasite directly on the living host. It is a well known fact that a parasite in pure culture on artificial media is made to lead a saprophytic mode of life and this condition often markedly alters its morphology. Again, not all parasitic fungi lend themselves readily to pure cultural methods, this being especially true with the obligate parasites. It is then highly important that we study ways and means of culturing parasites on the living host and of carrying continuously on the host such ones as are difficult or impossible to propagate on artificial media.

Little has been contributed on the culturing of parasites on the living host for extended periods of time. This has undoubtedly been due to the lack of facilities for attacking a problem of this nature. It is only within comparatively recent years that adequate greenhouse accommodations for botanical teaching and research have become a reality. Now that these are becoming more common it is important that we understand the growing of both parasitic and saprophytic fungi under greenhouse conditions so that we may always have at hand abundant living material, winter and summer, for the teacher and investigator. It is the results of my observations and experiments on this subject with special reference to plant parasites that I wish to discuss in this paper.

In attempting to culture a fungus on the living host, we are confronted with the same general considerations that are met in growing a fungus in pure culture on artificial media. It is not enough merely to have the host plants, they should be in a suitable container, at a susceptible stage, with environmental conditions favorable for both the host and the parasite, and with apparatus at hand for making transfers and inoculations.

DESCRIPTION OF APPARATUS

Flat and hood. The question of first importance in culturing plant parasites on the living host is a suitable receptacle for the host plants. In my earlier studies of Cystopus, it was found that large pots were better than small ones, in that more seedlings could be propagated on less area, a condition highly favorable for the fungus. Flats are even better than the large pots if one wishes simply to carry the fungus in culture for extended periods. For class use, however, the large pots

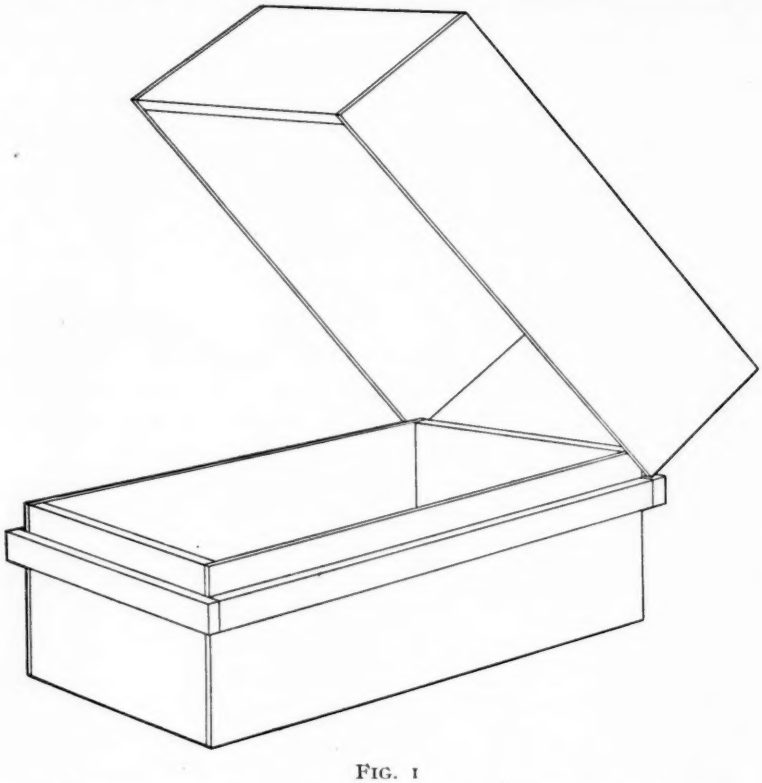

FIG. 1

are fully as convenient. The flats used are narrower and deeper than those commonly employed by the commercial florist. One made of cypress, 18 inches long, 10 inches wide and 6 inches deep has been found the most satisfactory (Fig. 1). These are filled with soil and autoclaved at 15 lbs. pressure for one hour before planting.

The scheme of using flats instead of pots, fortunately, makes it possible to dispense with bell jars, which are expensive and often inefficient. A wooden hood (Fig. 1) has been found the most simple and efficient means of controlling the humidity. It has inside dimensions in length and width corresponding to the outside dimensions of the flat

covered. The depth may be anywhere from 6 inches to several feet, although one 6 inches high has been found generally adequate, since seedlings are mostly used. The hood rests on a small ledge set three-fourths of an inch down from the upper edge of the flat, making the contact between the flat and hood very close so that no appreciable amount of evaporation can take place when the hood is in position.

Atomizer. Another piece of apparatus of great importance in maintaining cultures of fungi is some contrivance for distributing the spores upon the host tissue to be infected. As is well known, there is no piece of apparatus made especially for this purpose. Atomizers of various types are commonly used, although often with difficulty and inadequate results, which is not strange since these atomizers were

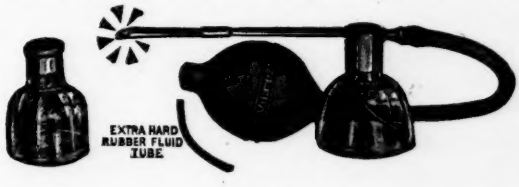

FIG. 2

originally designed to aid the physician. Some are better fitted for handling a suspension of spores than others. An atomizer in order to be well suited for infection experiments must be so constructed that it may (1), give either a coarse or fine spray without altering or readjusting any of its parts; (2), be easily taken apart; (3) be capable of sterilization; (4), transmit the spray in any desired direction by means of a movable nozzle; (5), and above all else, be easily cleaned when clogged. The instrument I have found most satisfactory, a De Vilbiss No. 16, is shown in Fig. 2. It is made by the De Vilbiss Manufacturing Company, Toledo, Ohio.

Refrigerator. The importance of controllable low temperature for spore germination and infection in certain cases has already been demonstrated.[1] I have found a large refrigerator with an ice capacity of 400 lbs. most satisfactory for this purpose. The temperature in this is quite constant, changing less than one degree in 24 hours. By varying the amount of ice, however, temperatures from 8° to 14° C. are easily obtained. In such a refrigerator, it is possible to place twelve hooded flats at the same time.

Humidity box. For temperature above 14° C. and not higher than 22° C. a humidity box is used. It is 4 ft. long, 3 ft. wide and 2 ft. high, having glass sides and top. It stands near a north window upon a table in an ordinary laboratory adjoining the greenhouse. The

[1] Melhus, I. E. Experiments on spore germination and infection in certain species of Oomycetes. Wisconsin Agr. Exp. Sta. Research Bul. 15: 25-91. 1911.

bottom of this box, which is of wood, is covered with two inches of wet moss. The temperature within is, of course, influenced by that of the laboratory. The humidity, when closed, ranges from 95 to 96 per cent, a condition highly conducive to germination and infection. It is not necessary, therefore, to use the hood previously described when cultures are placed in this box, since no appreciable amount of evaporation from the plants takes place.

CULTURAL STUDIES

Cystopus candidus. My method of culturing Cystopus on the radish has been described in detail in the publication already cited. The hosts were grown in 6 inch pots. The seedling plants were sprayed with a suspension of spores in water, then subjected to a saturated atmosphere at comparatively low temperatures for 10 to 24 hours. Following this treatment, the cultures were kept under favorable conditions for vigorous development of the host. Ninety per cent of the seedlings in each culture became infected by this treatment. The character and extent of infection can be seen by referring to the plates of the article already noted.

More recently, I have substituted small flats for the pots with even better results. The fungus fruits in from 6 to 10 days after inoculation, depending upon environmental conditions. Only conidia are produced on the cotyledons and leaves. Oospores are formed only when the flowers and fruiting organs of the host become infected.

Other species of Cystopus, *e. g. C. bliti* on *Amaranthus retroflexus* and *C. portulacae* on *Portulaca oleracea*, can be cultured in the same way, though with more difficulty, since these latter host plants are not as easily propagated as the radish during the late fall and winter.

Peronospora parasitica. In Wisconsin *Lepidium virginicum* is commonly and vigorously attacked by this Peronospora, the infection becoming systemic in many cases. The fungus is easily propagated during the winter on the Lepidium in the following manner:—

In the fall when the second crop comes up and becomes infected with Peronospora the plants can be dug up and brought into the greenhouse. Here they will continue growing throughout the winter. The fungus grows along in the young tissue of the host, although sporulating very sparingly in a dry atmosphere. Sporulation can be induced at any time by placing the infected plants in a saturated atmosphere either in the humidity box or any other contrivance affording a highly humid atmosphere. The Lepidium plants can be collected and transferred to the greenhouse not only in the fall of the year but at any time during the winter from the first frost until the ground thaws out in the spring. I collected them infected with Peronospora on 8 different dates between November 30, 1911 and March 25, 1912.

Each time the plants renewed activity and the Peronospora sporulated abundantly in 2 to 5 days. The fungus does not kill the host immediately and the same infected plants can be kept alive for three months. The host reacts to the fungus in much the same way as it does out of doors during the spring and summer. The leaves and stems become much enlarged and hypertrophied as well as chlorotic. Conidia are produced in abundance but oospores develop only sparingly in the dying tissues. No attempt has been made to culture *Peronospora parasitica* during the summer because of its great abundance on the Lepidium during this time of the year. It is very probable that other fungi with similiar habits can be cultured in this way.

Puccinia helianthi. The method used in culturing Cystopus has been applied with some modifications to *Puccinia helianthi* on *Helianthus annuus.* Sunflowers, like radishes, grow rapidly and thereby supply abundant food for the fungus in a short time, a condition highly essential. The rust sporulates freely and this makes its propagation readily possible. The sunflower rust has been carried in culture for the past two years. Plate XX, Fig. 2, shows the extent of infection maintained. This culture was started by planting the seeds January 4, 1912. The young plants were exposed to infection January 30 by spraying the foliage with a suspension of spores taken from a previously infected culture. It was covered with the hood and placed in the refrigerator at 14° C. for 24 hours. At the close of this period, the hood was removed and the flat returned to the greenhouse. Evidence of infection first began to appear on February 9, 10 days after being exposed to infection. The photograph reproduced in the plate was made February 14. Uredo- and teleuto-spores were produced on both surfaces of the leaves.

Puccinia coronata. Crown rust of oats has also been maintained in culture during the past two years. One would naturally expect that so vigorous a parasite would maintain itself under greenhouse conditions if simply supplied with host tissues, but this has not been my experience. In fact, this rust is more difficult to hold in culture than the sunflower rust just described. This is chiefly due to the less rapid growth of the host. The possibilities of the fungus vary directly with the amount of host tissue available, other factors being equal. The young seedlings were exposed to infection February 4 by spraying them with a water suspension of spores taken from a previously infected culture. The culture was then placed in the humidity box for 24 hours where the temperature was about 16° and the humidity 95 percent. By February 14 rust pustules began to appear very abundantly on the oat seedlings. It has required six cultures of oats

to maintain well-infected stock cultures of the crown rust. Reinoculations were made on the cultures once infected as soon as the extent of infection materially decreased, which was usually about every three or four weeks. Uredosori were produced in abundance on both leaves and young stems from 8 to 12 days after exposure to infection. Teleutospores develop about two or three weeks later.

Puccinia graminis. The rust on wheat has been cultured in the same way as the crown rust of oats.

Puccinia sorghi. Corn rust is more easily cultured than either of the cereal rusts. Intermittent exposure of once infected cultures has not been found necessary, although the fungus has been propagated winter and summer in the greenhouse. The cultures were started with spores taken from infected garden sweet corn. These were scraped from the infected leaves with a thin-edged scalpel and placed in two ounces of water in a DeVilbiss atomizer. The corn plants were sprayed with the spore suspension, covered with the hood, and subjected to 18° C. for 24 hours. At the end of this period, the seedlings were uncovered and returned to favorable conditions for growth of the host. Infection was evident eight days later. The amount of infection increased as the corn plants grew larger. By keeping six cultures of corn growing vigorously in close proximity to one another, the rust easily spreads by natural agencies and has thus been maintained continuously for two years. Only uredosori developed on the corn plant indoors, even though the host plants were allowed to remain in the cultures until dead.

Cercospora beticola. During the late summer and fall of 1911, the Cercospora leaf spot developed very abundantly on a small plot of mangels in our pathological garden. This led me to test the possibility of culturing this fungus on the living host under greenhouse conditions. Other mangels were sown and when they were about 6 inches tall, they were exposed to infection by spraying them with a suspension of viable spores taken from the infected plants referred to above. The young plants were placed in the humidity box for 24 hours after being sprayed with the suspension of spores. The typical Cercospora spot began to appear October 20. Sporulation followed as the spots became older. It was necessary to re-expose the cultures to infection by spraying them again with a water suspension of spores on November 10, December 27 and February 14. Since the last date, the fungus has required no assistance in maintaining infection. It is evident from the above data also that the chances of the leaf spot increased directly, under the existing greenhouse conditions, with the amount of host tissue available for infection by the fungus. Conidial fructification was faciliated by placing the cultures in the humidity box for 24 hours.

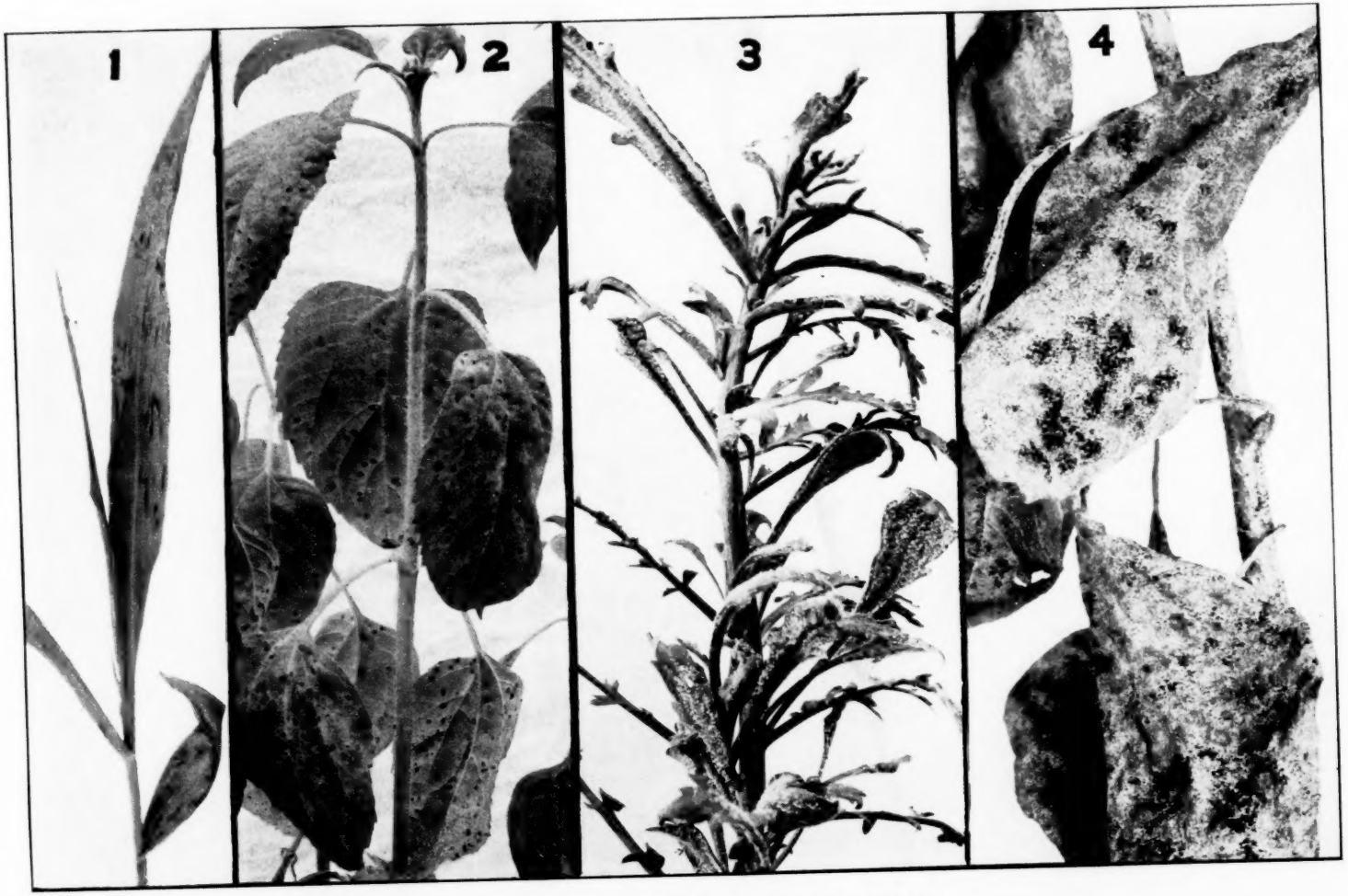

Plate XX.—Cultures of Fungi on Living Hosts

Erysiphe cichoracearum. It is sometimes possible, if one so desires, to culture two fungi on the same host at the same time. Such is the case with *Puccinia helianthi* and *Erysiphe cichoracearum.* In the fall of 1911 when the powdery mildew was abundant out of doors on the cultivated sunflower viable spores were collected and sunflower seedlings were exposed to infection by spraying them with spores. Four cultures were so treated, two that were already infected with Puccinia and two that were not. The mildew began to appear on the sixth day. No difference was noted as to the extent of infection developing on the clean and rusted seedlings. Gradually the mildew spread over both sides of the leaves and stems until the fungus was evident at a considerable distance. It was not necessary to reinfect either the old cultures or new ones. The fungus maintained itself when susceptible host tissue was supplied in abundance. The culture of this species of Erysiphe is of particular interest because it produces not only conidia but also abundant perithecia. In the cultures referred to above, the perfect stage of the fungus became very abundant when the host tissues began to die, the leaves and stems being literally covered with perithecia. (Plate XX, Fig. 4). The fact that this species of Erysiphe produces perithecia so profusely and is so cosmopolitan in regard to the host species attacked, makes it a very favorable form for teaching purposes.

Erysiphe graminis. The culture of *Erysiphe graminis* on the various cereals under greenhouse conditions has already been worked out by Reed.[1] Initial infections were obtained on seedlings growing in large pots. The fungus after this maintained itself when supplied with new seedlings as the old were killed. In the same way as explained above, except flats were substituted for pots, I have been able, for the past two years, to carry cultures of *Erysiphe graminis* on three cereals: wheat, rye and barley. The fungus produces great quantities of conidia, but no perithecia, in culture. This abundance of conidia, together with the marked development of physiological specialization, makes it a very interesting form to combine with *Erysiphe cichoracearum.*

DEPARTMENT OF PLANT PATHOLOGY,
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EXPLANATION OF PLATE XX

Fig. 1. One of the corn seedlings taken from a culture of *Puccinia sorghi.*
Fig. 2. A plant of *Helianthus annuus* showing the extent of rust infection maintained in culture.
Fig. 3. *Lepidium virginicum* infected with *Peronospora parasitica* in culture.
Fig. 4. *Erysiphe cichoracearum* on *Helianthus annuus*; advanced stage showing abundant perithecia.

[1]Reed, George M. Infection experiments with Erysiphe graminis D. C. Trans. Wis. Acad. Science, Arts and Letters. 15: 135–162. 1905.

THE CHESTNUT BLIGHT FUNGUS AND A RELATED SAPROPHYTE

P. J. ANDERSON AND H. W. ANDERSON

Early in our investigation of the chestnut tree blight it was noticed that the fungus in the southwestern counties of Pennsylvania showed marked differences from that in the eastern part of the State. The most striking of these were that it rarely if ever grew as a parasite on living trees and that the typical fan-shaped areas of mycelium in the bark, characteristic of the eastern fungus, were absent. It is the purpose of the writers in this article to make clear the relation between these fungi. The external similarity of these species has given rise to a certain amount of confusion as to the extent of the blight. We shall first show in detail the differences between the fungus known as *Diaporthe parasitica* and the one in western Pennsylvania known as the "Connellsville fungus." The nomenclature of these and other related fungi will then be discussed.

Morphological differences. The principle morphological difference between the Connellsville fungus and *Diaporthe parasitica* is in the size of the asci and ascospores and in the shape of the ascospores. Our measurements are based on the examination of a large number of specimens collected in western Pennsylvania, Virginia, West Virginia and Tennessee. Measurements of several thousand ascospores from widely scattered localities gave an average of $7 \times 2.98\mu$ for the Connellsville fungus. The asci averaged 34μ in length. The measurement of an equal number of ascospores of Diaporthe from various counties of eastern Pennsylvania and New York State, show an average size of $8.6 \times 4.5\mu$, while the asci have an average length of 51.3μ. All measurements were made with a $\frac{1}{12}$ oil immersion. The contrast in the size here given is striking, but even more striking is the difference in the shape of the ascospores. As shown by the measurements, the Diaporthe ascospores are much wider in proportion to their length than those of the Connellsville fungus. The relation is about 1 : 1.9 in the former and 1 : 2.7 in the latter. Furthermore, the septa in Diaporthe are very evident and a distinct sinus may be seen in the mature spores while an indistinct septum and a very slight sinus, if any, is the rule in the Connellsville fungus. These characteristics are so evident that a glance at the spores under the microscope by one familiar with the two fungi is sufficient to distinguish them provided the ascospores are mature. The perithecia of Diaporthe are also much larger than those of the Connellsville fungus. On account of the very small size of the conidia no attempt was made to distinguish between these.

The constant difference in the size and shape of the ascospores and asci seems to us to be sufficient basis for a specific separation of these two fungi. But we would hesitate to make this separation were not marked differences found in the cultural and pathological characters of the two.

Cultural differences. Both of these fungi produce conidial spore horns in much the same manner. If these spore horns are streaked on a potato agar slant, Diaporthe will produce an orange streak within four days at room temperature. This orange streak broadens, keeping pace with the growth of the fungus until the entire surface of the slant is covered with a deep orange growth. On the other hand no orange color is noticeable on the streak from the conidia of the Connellsville fungus even after a period of ten days. A lighter orange sometimes develops on these slants after a week or so but it is never so marked as from Diaporthe, and often fails to develop at all. Conidial streaks on other media, especially chestnut bark agar and corn meal agar, show a recognizable difference between the two fungi but this difference is not so marked as that of the color on potato agar.

On potato agar cultures from mycelial transfers a fan-shaped or irregular wavy growth is noticable at the edge of the advancing mycelium in the case of Diaporthe while the Connellsville fungus has an even, unbroken edge. Furthermore, there is a marked contrast in the amount of aerial mycelium developed, Diaporthe developing scarcely any while the Connellsville fungus has a fluffy appearance due to a white mycelial growth above the surface of the agar. Also the contrast in color between the growths on potato agar is evident, especially in cultures about three weeks old. Diaporthe develops an orange-brown color while the Connellsville fungus has at first a sulphur-yellow color which deepens as the culture becomes older.

Next to the conidial streaks on potato agar, we have found the growth on sterile twigs in test tubes to be the most accurate distinguishing character. The Connellsville fungus within ten days develops a fluffy orange mycelial growth which almost completely fills the tube. This mycelial growth is at first white but turns to the orange color within a few days after its development. On the other hand, Diaporthe does not develop this heavy aerial mycelium but only a short, white, web-like growth over the surface of the twig with heavier bunches of mycelium, which later become orange colored, where the pycnidia are to develop. On the cut ends of the twigs Diaporthe develops a thick felt-like orange mycelial growth, but this never extends out on the bark and is much denser than the growth of the Connellsville fungus. We have made these cultures on black oak, chestnut

oak, white oak, chestnut, maple and sumach, but find very little difference in the nature of the growth. We have used these tests on fungi from over fifty different sources and have never failed to get these characteristic reactions. These tests are always checked when possible by ascospore measurements and often by inoculation on live trees. No doubt many other cultural differences could be discovered by further tests but these given have proved to be so reliable that no further effort was made to find media which would show additional differences.

Pathological differences. The question whether the Connellsville fungus is a parasite or a saprophyte is one of paramount importance to those interested in the chestnut blight investigation. It was for the purpose of determining this point that these investigations were undertaken and as a result we are now able to say with certainty that the Connellsville fungus is not an active parasite. We base this statement on the following facts: (1) We have never found a tree killed by this fungus although we have examined thousands of trees in the region where it is extremely common on dead stumps and logs; (2) our inoculation experiments prove that it will not grow as an active parasite on the living portion of the tree. In a number of cases we have found this fungus growing on live trees but it was either following some other disease or injury, or growing on the dead bark above the living part of the tree and had not penetrated into the cambium. In the latter case it was usually found on old trees with thick heavy bark. In several instances we have isolated it from the edge of what appeared to be a canker on young coppice but as a rule by careful examination insects or other fungi could be found as the primary cause of the canker. This fungus may under certain conditions prove to be a weak parasite but there is no reason to believe that it ever was or ever will be an active destructive parasite like *Diaporthe parasitica*.

Inoculations with Diaporthe both at Connellsville and at Charter Oak produced the disease with all its typical symptoms within a month. Over five hundred inoculations made with the Connellsville fungus at the same points under the same conditions gave uniformly negative results. The fungus developed in the dead tissue above the wound caused by the inoculation and in many cases spore horns were produced. This shows that the inoculations were successful in that the fungus continued to live and grow after being introduced. It also shows that the conidia and ascospores germinated and produced mycelial growth in the dead tissue. There was however, in no case any indication of the fungus attacking the live tissue and in all cases the callus was formed at the edge of the wound and in inoculations

made three months ago the wound has almost healed over. There is always a definite even line separating the dead brown bark and the living tissue which is very different from the scalloped edge and the invading fan-shaped mycelial areas of the Diaporthe canker.

Distribution of the Connellsville fungus. When a fungus was collected from a new locality it was subjected to all the cultural tests given above, and if the ascosporic stage was present spore measurements were made. In this way there was no danger of confusing the species even if they happened to be in the same locality. Our collections indicate that this fungus is distributed over southwestern Pennsylvania, West Virginia, Virginia and eastern Tennessee. It is probable that it will be found more or less throughout the eastern States and southward. It has been collected on various oaks and on the chestnut. It is most abundant on the stumps of chestnut from which the trees have been cut one or two years.

There is another fungus found in the extreme south—Florida, Alabama, South Carolina and Mississippi—which is very similar in external appearance both to the Connellsville fungus and to Diaporthe. This is the fungus found in Ellis and Everhart's N. A. Fungi (No. 1956), where it is labelled *Endothia gyrosa*. It is also found in a number of other North American collections under this name. The ascospores of this fungus measure 8.2 x 1.90 μ, being much longer in proportion to their width than the Connellsville fungus. They are cylindrical in shape and are very well illustrated in Ellis and Everhart's North American Pyrenomycetes. Besides the exsiccati we have also received specimens of this fungus from several points in North and South Carolina. One of these, collected by Dr. J. G. Hall, of Clemson College, South Carolina, and sent to us by Dr. Haven Metcalf was identified by Dr. C. L. Shear as *Sphaeria radicalis* (Schw.).

Taxonomic relations. What is the Connellsville fungus? There is no question but that it is very closely related to *Diaporthe parasitica* and should be placed in the same genus. Following Saccardo's system of classification it undoubtedly falls in the genus Endothia and fits well his description of *Endothia gyrosa* in so far as the spore measurements and macroscopic characters are concerned. Whether it is the same fungus as that occuring in Italy and called *Endothia gyrosa* we cannot say until we have examined either the ascospores or cultures from that country. It is certain, however, that it is not the same as the long-spored southern form identified as *Sphaeria radicalis*.

The synonomy of *Endothia gyrosa* given by Saccardo is misleading since it is certain that Schweinitz and Fries had in mind two very different species when they wrote of *Sphaeria gyrosa* and *S. radicalis*.

Furthermore, if the genus Endothia was founded by Fries on *Sphaeria gyrosa*, as Farlow[1] believes it was, then the generic name Endothia is not correct when applied to this fungus—provided we go back to Fries[2] for the definition of the genus.

Schweinitz[3] in 1822 described *S. gyrosa* as No. 24 of his Syn. Fung. Car. but so far as we have been able to find there is no specimen in the Schweinitzian collections corresponding to the number of this description. It is probable that the specimens of this collection were included in his North American Fungi and in this collection there is a specimen of this species (No. 1431) which fits very accurately his description of *S. gyrosa* in Syn. Fung. Car. In fact, in looking at this specimen under a powerful lens one is struck with the extreme accuracy of his description and one cannot doubt that he had this or a similar specimen under his lens. This fungus is entirely different from *Diaporthe parasitica* or the Connellsville fungus and one would not think of placing it in the same genus or even in a related genus. The most noticeable character macroscopically is that the entire surface of the stroma is covered with very regular hemispheres (sphaerulae of Schweinitz). The perithecia are enclosed in each of these separate sphaerulae and have no distinct wall, *i. e.*, it can not be differentiated from the surrounding stroma. No neck is evident and the ostiole is inconspicuous or wanting. The conspicuous black walls and long necks of the perithecia of Diaporthe are entirely lacking. The perithecia are entirely within the knobs or spheres of the stroma, *i. e.*, they do not extend down into the stroma. The ascospores are often slightly curved and the septa very indistinct. The average size of the ascospores was 15.82 x 4.37μ. The asci have very distinct walls and are not shaped at all like the asci of Diaporthe. They average 54μ in length.

Schweinitz's[4] *Sphaeria radicalis* (N. A. Fungi No. 1269) is an entirely different fungus from the above and although the perfect stage is not present, it resembles very closely the imperfect stage of either the Connellsville fungus, *Diaporthe parasitica* or the long-spored southern form. Any one who has worked with the above species will be convinced that Schweinitz was writing of the perfect stage of one of these forms when he says, "Ostioles cylindrical, very black within, orange red externally, everywhere elevated on the surface,

[1] 1912. Farlow, W. G. The fungus of the chestnut tree blight. Sci. N. S. 35 : 717–722. May 10, 1912.

[2] 1846. Fries, E. Summa vegetabilium Scandinaviae, p. 385. 1846.

[3] 1822. Schweinitz, L. D. Synopsis Fungorum Carolinae, p. 3. 1822.

[4] 1831. Schweinitz, L. D. Synopsis Fungorum in America Boreali media degentium, p. 197. 1831.

easily falling off—*whence the exposed surface shows black points, on account of the black-shining ducts by which the ostioles are connected with the perithecia.*" But which of the three he had in mind would be hard to say unless the perfect stage is examined. Fries[4] places *S. gyrosa* in the tribe Confluentes and *S. radicalis* under the tribe Versatiles, thus widely separating the two since the characters of these tribes are quite distinct. His descriptions of the two species follow closely those of Schweinitz. His distinction between the two is best brought out in his Elenchus[5] where, in describing *S. radicalis*, he says, "A wonderful little fungus—certainly comparable only with *S. gyrosa* but very *different from this in the position of the perithecia and the ostioles.* Ostioles numerous, conical, elongated, fragile, spine-like. Perithecia minute, black, globose, sunken, also continuous through the spine-like ostiole by a little black duct." Under *S. gyrosa* he says, "There is no distinct ostiole," and does not mention the beaks so noticable in *S. radicalis*. This agrees well with the specimen of Schweinitz, where no ostioles are to be seen and the small knobs on the surface of the stroma contain simply the perithecia with no distinct necks.

It is, therefore, evident that Fries had clearly in mind the distinction between these two species when he created the genus Endothia in 1846. In this meager and incomplete description he does not mention *S. radicalis* but he does mention *S. gyrosa* and it is to be presumed that he intended this species to be the type of the genus. Although he promised to describe more fully the characters of this genus later, we find no further mention of it in his later publications. If we admit that he used *S. gyrosa* as the type for erecting this genus and if we wish to include under it only species resembling it, then it is evident that the present Endothia is an entirely different genus from what Fries intended. As further evidence that he did not intend to place *S. radicalis* in this genus we may turn again to his description where he speaks of the perithecia being light colored (pallidus) and yet he distinctly mentions the dark (nigra) walls of the perithecia in his description of *S. radicalis*.

In 1863, however, DeNotaris[6], gave an accurate and fairly complete description of the genus Endothia based on the *Sphaeria radicalis* of Schweinitz. He nowhere mentions *E. gyrosa* but refers to the description of the genus by Fries. It is therefore certain that he either considered the reference of the latter author to *S. gyrosa* as without significance or he regarded the two species—*S. radicalis* and *gyrosa*—

[5] 1828. Fries, E. Elenchus Fungorum, **2** : 73. 1828.

[6] 1863. DeNotaris, G. Sphaeriacei Italici. Cent. I, Fasc. 1, p. 9. 1863.

as synonyms. In the same year we find them combined by Tulasne[7] under the genus Melogramma. Since that time all authorities without further investigation have considered that Schweinitz gave these two names to one and the same species.

If the generic name Endothia is to be retained for those species resembling *S. radicalis* of Schweinitz and Fries and the more recently described *Diaporthe parasitica*, then we believe that the Connellsville fungus would fall in the genus Endothia. If, however, we wish to retain under this name such species as that on which Fries erected the genus, the Connellsville fungus would certainly not fall in this genus and a new one will have to be erected to include *Diaporthe parasitica*, the Connellsville fungus and the long-spored southern form of Ellis and Everhart. According to our present system of classification, the form on which Fries erected the genus Endothia would easily fall in a previously established genus and this name is now left without any significance whatever. Besides, we are not certain that Fries meant to give a generic description in this short note since he states that he expects to describe the genus more fully later. The simplest way out of this taxonomic tangle then, it seems, would be to retain the name Endothia for the forms such as Saccardo includes under it. Then we would have in our territory (1) *E. radicalis* (Schw.) Fr., (2) the true blight fungus—why not call it *E. parasitica*—and (3) the Connellsville fungus, for which we propose the name *E. virginiana* and for which we expect to write a description as soon as more of the European specimens have been examined.

PATHOLOGICAL LABORATORIES OF THE PENNSYLVANIA
CHESTNUT TREE BLIGHT COMMISSION

[7] 1863. Tulasne. Selecta Fung. Carpologia. Tome II : 87. 1863.

THE CHESTNUT BLIGHT FUNGUS

C. L. SHEAR

During the past few months the writer has had opportunity to examine numerous specimens of the *Endothia radicalis* of European authors. He has also collected an abundance of fresh material of the same fungus on the chestnut in Italy and Switzerland. Cultures have been made from this material and also inoculations of American chestnuts. Owing to the wide spread interest in this subject, a brief statement of the present status of our investigations may be justified. Our studies of European material have convinced us that the so-called *Endothia radicalis*, found on the chestnut in Europe, is identical, morphologically, with *Diaporthe parasitica*, Murr. as found in America. The cultures and inoculation experiments have not been carried far enough at present to justify positive statements in regard to the physiological features of the organisms. No evidence of active parasitism on the part of the European fungus could be found on the chestnut in Italy and Switzerland. The Endothia was common in northern Italy and southern Switzerland and was almost invariably found on bark about the top of stubs and stumps which had been cut apparently from nine to eighteen months previously.

We have found as yet no European specimens of typical *Endothia radicalis* (Schw.). Schweinitz's species is fairly well represented by Ellis and Everhart in North American Pyrenomycetes, Plate 30, Figures 6 to 8, though the ascospores frequently show a greater tendency to curve and the indication of the division near the center of the spore is frequently wanting. We have seen as yet no intergrading forms between the true *Endothia radicalis* (Schw.) and *Diaporthe parasitica* Murr.

There is probably no specimen of Schweinitz's *Sphaeria gyrosa* extant which shows ascospores, except a collection which Schweinitz referred to this species long after the original description was published. This is apparently a Nectria. The specimen of this species which Schweinitz sent to Fries and also at least part of that sent to Schwaegrichen appear to show pycnidia only, though we were unable to make a microscopical examination of the Friesian specimen preserved at Upsala, upon which the genus Endothia, in part at least, was founded. The specimen apparently used by Fries as a basis of his description was so small that Professor Juel, who has charge of Fries' herbarium did not think it advisable to cut it. So far as could be determined with a hand lens this specimen appeared identical with one found in Schweinitz's herbarium at Philadelphia and also in the herbaria of other

mycologists to which he had sent specimens. These specimens have thus far shown only pycnidia and pycnospores and their identity with *Sphaeria radicalis* Schw. is, therefore, somewhat doubtful.

The European Endothia is evidently an indigenous fungus and has been known for nearly one hundred years. There is little doubt that *Sphaeria fluens* Sow., described and figured by Sowerby in the supplement to his English Fungi, 1814, Plate 420, published as part of Plate 438, from a collection made by Charles Lyall in the New Forest of southern England, is the pycnidial condition of *Endothia radicalis* DeNot. Through the courtesy of Sir David Prain, Director of the Kew Botanical Garden, we have been permitted to study Sowerby's specimen, as well as others preserved at Kew.

As a result of our studies to date we are of the opinion that *Diaporthe parasitica* Murr. is the same as *Endothia radicalis* of European authors, but not of Schweinitz, and that it was probably introduced into this country from Europe and has gradually spread from the original point of introduction, its spread being facilitated chiefly by borers or other animal agencies which produce wounds favorable for infection by the fungus. That *Diaporthe parasitica* is not a native fungus seems probable from the fact that no specimens of it had been collected, so far as we have been able to learn, previous to its discovery near New York less than ten years ago. If the fungus had been present here before, even as a saprophyte, it does not seem likely that it would have escaped detection by Schweinitz, Ellis, Peck, or some of the many other mycologists and collectors who have worked in the region. Its apparent spread from a single center also suggests that the organism has been introduced. Its attack on the American chestnut may be due to the greater susceptibility of our species than its native European host. It is hoped to publish a full account of our studies later.

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PHYTOPATHOLOGICAL NOTES

Isolation of pathogenic potato bacteria: A question of priority. Dr. Julius Schuster's statement in his historical review of potato diseases (*Zur Kenntniss der Bakterienfäule*, p. 453) that Frank was the first person who had *pure* cultures of a pathogenic potato bacterium needs only a slight emendation to approximate historical accuracy. It should read: *Frank war somit der Erste* [*im Deutschland*] *der ein Kartoffelpathogenes Bakterium in rein* [*?*] *Kultur hatte.* Something also is wrong with a historical sketch which devotes two pages to a variety of inconclusive and erroneous observations and only two lines to the first paper (26 pp.) on a bacterial disease of the potato, worked out according to modern bacteriological methods, and the stated conclusions of which remain uncontradicted after a lapse of eighteen years. It is not necessary to write historical résumés, but if one undertakes to do so he should state things as they are. The first person who had pure cultures of a pathogenic potato bacterium was an American, Prof. T. J. Burrill (Univ. of Illinois, 1890–91) and the second person was also an American, viz, the writer of this note (U. S. Dept. of Agriculture, 1895–96).

The writer's bulletin, U. S. Dept. Agr., Div. Veg. Phys. and Path., No. 12, on the bacterial brown rot of tomato, eggplant, and Irish potato, was published in 1896. The same year in his handbook, *Die Pilzparisitären Krankheiten der Pflanzen*, Frank denied the existence of bacterial diseases in general and of bacterial potato diseases in particular. Any one who will take the trouble to compare the fragmentary and misleading statements in Frank's paragraph on wet rot of the potato in this book (pp. 21–23) with my own statements in bulletin 12, the result of numerous successful pure culture inoculations covering a period of two years, will then know how the situation lies historically. I sent Frank a copy of this bulletin and the following year (1897) he recanted, as the result, he said, of his own observations, but we do not know to this day and never shall know what his *Micrococcus phytophthorus* really represents, or whether he ever had any pure (unmixed) cultures of a bacterium pathogenic to potato. Schuster may think so, but where is the proof?

Several hypotheses lie open to us, any one of which may be the true one:

(1) Frank once had from black leg of the potato a pathogenic micrococcus which nobody since has been able to find or would be able to identify with his *Micrococcus phytophthorus*, if found.

(2) Frank had under observation what we now know as *Bacillus phytophthorus* and mistook it for a coccus; or found a coccus and supposed he had the parasite, *i. e.* as Appel suggests, generalized a single observation.

(3) Frank did not carefully control his cultures and as a result sometimes he had pure cultures of a micrococcus which was not infectious; and sometimes mixtures of bacilli and micrococci which were supposed by him to be pure cultures of *M. phytophthorus*, and which were infectious because they contained, among other things, what we now know, through the distinguished labors of Appel, as *Bacillus phytophthorus*.

So far from clearing up the situation by his labors, Frank misled everybody who put any trust in them, not only with respect to potato diseases but all other bacterial diseases.

Finally, it may be pointed out once more that eight years prior to Frank's publication, Prof. T. J. Burrill isolated from rotting potatoes in pure culture a bacterial organism which he proved two years running by numerous inoculations to be infectious to leaves, stems and tubers (Proc. Soc. Prom. Ag. Sci., 11th Ann. meeting, 1890, pp. 21–22; 12th Ann. meeting, 1891, p. 29.)—ERWIN F. SMITH.

A New Method in Bacterial Research. In the August (1912) number of the Journal of Experimental Medicine, Churchman gives a new test of unusual interest corresponding to and supplementing Gram's stain. This he has tried on more than 130 species with striking results. For details the reader will consult the original paper; here may be mentioned, however, a few of the striking features. The test depends upon the fact that some schizomycetes absorb gentian violet readily and are killed by it, while others grow in its presence readily unless the dose is excessive. Various methods of applying the test are described. One of the simplest is to divide an agar plate by means of a strip of metal (or paper) into two portions. On one side is poured the ordinary nutrient agar and on the other side agar to which a small quantity of Grübler's gentian violet has been added (1 : 100,000, *i. e.*, about one 2-mm. loop of a saturated water solution to each 10 c. c. of the melted agar). After the agar has hardened and the strip has been removed the organisms to be tested may be streaked across the two agars putting equal amounts on the two sides. Violet *positive* organisms grow only on the unstained agar and generally not quite up to the dividing line while violet *negative* organisms grow on both agars; *e. g.*, *B. subtilis* grows only on the unstained agar, while *B. typhosus* grows equally well on the stained agar. Numerous interesting figures and statements are given, *e. g.*, a mixture was made of *B. typhosus* with half a dozen violet-positive organisms and, when plates were streaked, a mixture developed on the ordinary agar, but only a pure culture of *B. typhosus* on the gentian-violet agar. Again, a culture of *B. subtilis* supposed to be pure, when it was streaked on

these plates developed a pure culture of *B. coli* on the stained side, showing unsuspected contamination, not easily detected in other media owing to the overgrowth of the *B. subtilis*.

In general, organisms that stain by Gram were found to be violet positive, but the two tests were not altogether parallel.

It seems likely that this test will soon take its place among the regular and necessary laboratory tests of microorganisms.

A preliminary test in this laboratory has shown the following reactions:

Violet positive.	*Violet negative.*
B. omnivorus +	Schiffs olive organism —
B. amylovorus +	B. beticolum —
B. phytophthorus +	B. melonis —
B. solanisaprus +	B. xanthochlorum —
B. melanogenes +	B. oleraceae —
B. savastanoi +	B. stewarti —
B. subtilis +	B. carotovorus — (not a good growth.)

ERWIN F. SMITH

A Botrytis on Conifers in the Northwest. During the summer and fall of the present year, a serious fungous disease affecting only the young terminal shoots, mostly of the Douglas fir has been noted in the Priest River Valley, Idaho (Kaniksu National Forest). The rainfall in this region during the present season has been unusually heavy, with alternate periods of warm foggy weather. On the low river flats the young Douglas fir frequently occurs in close, even-aged stands, more often, however, in the poorer soils on the slopes of the deep elevated ravines. Usually the morning fogs remain suspended in these lateral valleys well up into the day. These factors afford favorable conditions for the development and spread of the fungus, which has proven to be a species of *Botrytis*. In its method of attack and subsequent behavior it closely resembles the form *Botrytis Douglasii* as described by von Tubeuf. Late in the season the affected needles produce small black sclerotia in abundance. Numerous fertile ascending hyphae from which ovoid conidia are produced appear on the diseased material when placed in a damp chamber. A few experiments showed the parasitic nature of the fungus and that it was by no means confined to the Douglas fir. The young shoots of *Abies grandis*, *Tsuga heterophylla*, and young seedlings of *Larix occidentalis* were frequently found infected. The disease could also be produced in these species by inoculation from the Douglas fir. I have called attention to this disease, owing to its frequency in the forests of this region. It retards the growth of young conifers by killing back the tender shoots. The disease could easily become a serious menace to the nurseries located in this valley, if the present weather conditions should prevail.

JAMES R. WEIR

J. G. Grossenbacher has resigned his position as associate botanist in the New York Experiment Station to become pathologist in the Office of Fruit Disease investigations of the Bureau of Plant Industry. His place at Geneva is taken by W. O. Gloyer, formerly assistant botanist at the Ohio Experiment Station. Dr. R. Kent Beattie, recently professor of botany in the State College of Washington, Pullman, Wash., has accepted a position as expert in the Office of Forest Pathology of the Bureau of Plant Industry. Dr. Neil E. Stevens, recently assistant plant pathologist in the Kansas Experiment Station, has accepted a position as forest pathologist in the same office. He is succeeded at Kansas by R. A. Jehle, recently fellow in Plant Pathology in Cornell University. Dr. F. D. Heald has resigned his position as professor of botany in the University of Texas, to take charge of certain cooperative work on the chestnut bark disease maintained jointly by the Bureau of Plant Industry and the Chestnut Tree Blight Commission of Pennsylvania. His address is 1112 Morris Bldg., Philadelphia, Pa. Dr. A. F. Blakeslee has a year's leave of absence from the Connecticut Agricultural College (Storrs, Conn.) He has a temporary appointment on the staff of the Carnegie Station for Experimental Evolution at Cold Spring Harbor, Long Island, N. Y., where he will spend the year in research work on the Mucors. Prof. Geo. R. Lyman, of Dartmouth College assumes charge of the work of Prof. Roland Thaxter at Harvard University during the coming year, which is Prof. Thaxter's sabbatical year. D. F. Fisher has been appointed scientific assistant in the Office of Fruit Disease Investigations of the Bureau of Plant Industry. Miss Anna E. Jenkins, recently assistant in plant pathology in the New York College of Agriculture, has been appointed scientific assistant in the Pathological Collections of the Bureau of Plant Industry.

The International Congress of Comparative Pathology is to be held in Paris, October 17 to 23, 1912. This Congress has a section on Plant Pathology. American phytopathologists are invited to attend and submit papers. Titles should be sent to M. D. Brocq-Rousseau, Place Carnot, Nancy, France.

The National law regulating importation of nursery stock, etc., generally known as the Simmons Bill, was signed by the President August 20, and becomes effective October 1, 1912. An account of this law and its provisions will shortly appear in PHYTOPATHOLOGY.

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